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(57) Abstract

Infusions comprising a neuroprotectant and a thrombolytic agent as a combined preparation for simultaneous, separate or sequential use in the treatment of acute ischaemic stroke, and with the use of neuroprotectant for the preparation of a medicament for the treatment of acute ischaemic stroke and with the use of a neuroprotectant for the preparation of a medicament for preventing or delaying the process of infarction accompanying acute ischaemic stroke from being completed, thus enlarging the inclusion period during which the above combined preparation can be administered safely.

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INFUSIONS OF NEUROPROTECTANTS AND THROMBOLYTIC AGENTS

The present invention is concerned with products comprising a neuroprotectant and a thrombolytic agent as a combined preparation for simultaneous, separate or sequential use in the treatment of acute ischaemic stroke; with the use of such products for the preparation of a medicament for the treatment of acute ischaemic stroke and with the use of a neuroprotectant for the preparation of a medicament for preventing or delaying the process of infarction accompanying acute ischaemic stroke from being completed, thus enlarging the inclusion period during which the above combined preparation can be administered safely.

In US-4,861,785 there are described benzoxazol- and benzothiazolamine derivatives having anti-hypoxic and anti-anoxic activity. In WO-92/14,731 some of these benzothiazolamine derivatives were disclosed having useful anti-stroke activity. Injectable formulations of (S)-4-[(2-benzothiazolyl) methylamino]-α-[(3,4-difluoro-phenoxy)methyl]-1-piperidine-ethanol (generically known as lubeluzole) are disclosed in PCT/EP95/04520. Infusions comprising a neuroprotectant and a perfluorochemical as a combined preparation for simultaneous separate or sequential use in the treatment of conditions involving cerebral hypoxia are disclosed in European Patent Application No. 95202888.4 filed October 25, 1995.

The treatment of acute ischaemic stroke currently consists mainly of neuroprotective and of haematologic therapeutic strategies. As described in Cerebrovasc. Dis. 1995 ; 5 (suppl 1) : 27-30, though there is an increasing belief in the benefit of combined therapies for acute stroke treatment, optimal combinations remain to be determined. Such an optimized combination for the treatment of hypoxia is the subject of the present invention. In particular, it concerns a combination of neuroprotectant agent and a thrombolytic agent. The product is adapted to intravenous (or intra-arterial) administration by infusion as this represents the most appropriate route of administration for stroke patients.

Thrombolytic agents such as pro-urokinase and tissue plasminogen activator (t-PA) are currently in the final stages of clinical developments with a view to the approved therapeutic use of these thrombolytic agents in acute ischaemic stroke (The New England Journal of Medicine, 333 (24), 1581-1587, December 14, 1995).

The therapeutic utility of thrombolytic agents in the treatment of acute ischaemic stroke is severely limited by the fact that their administration must be effected within a short, limited period of time following the ischaemic event. This limited period of time is known in the art as the so-called 'inclusion period' during which stroke agent patient can be included in the group of patients who can be treated safely with a thrombolytic agent.

In clinical practice, patients who have suffered an ischaemic event will be screened before they are treated with a thrombolytic agent. This screening typically involves recording a computer tomographic (CT) scan with the purpose of determining whether or not the patient has any detectable brain damage, a so-called hypodense area or hypodensity which is indicative of intracranial haemorrhage.

Those patients who are diagnosed as positive in the screening will be excluded from receiving thrombolytic treatment. The underlying reason for this exclusion is that treatment with a thrombolytic agent will almost certainly be detrimental to the health of the patient, as the thrombolytic agent will cause thrombolytic recanalisation of the blood flow and subsequently cerebral haemorrhage (intracranial bleeding).

In any event, whatever the outcome of the screening test on the patients, current clinical practice is such that all practitioners will exclude all patients when more than a limited number of hours have passed since the occurence of the ischaemic event. For example, in the case of thrombolytic treatment with tissue plasminogen activator (t-PA), the 'inclusion period' is presently limited to three hours. Obviously, it is hoped that evidence will accumulate which will allow to prolong the 'inclusion period', for example to four, five, conceivably even six hours.

It is the present inventors' belief that a particular contribution to achieving the above defined goal can be realised by a specific combined treatment with a neuroprotectant, for example, with lubeluzole or a functionally analogous compound.

As a first consideration, it should be noted that neuroprotectants such as lubeluzole do not suffer from the limitations characterizing thrombolytic agents in the treatment of ischaemic events involving cerebral hypoxia. Thus, there is no need to screen patients beforehand (although such may well be advisable), before initiating treatment of a stroke victim with a neuroprotectant. The underlying reason for this is that neuroprotectants act by preventing or delaying the occurence and completion of neurological damage in occluded arterial blood vessels.

In the event of sudden revascularisation or recanalisation of an occluded blood vessel, the neuroprotectant will actually prevent, attenuate or delay the catastrophic event which is known as reperfusion damage. Reperfusion damage is the detrimental effect which follows the removal of, for example, a blood clot by a number of biochemical agents released by damaged (e.g. hypoxic) tissue at the site of infarction.

In summary, it is important to note that patients suffering from acute ischaemic stroke can be treated safely without prior screening from the first instance a physician attends to such a patient. Clearly, neuroprotectant treatment therefore is a first-line treatment for patients suffering from acute ischaemic stroke.

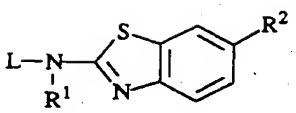
As a second consideration, it is now the inventors' belief that the previously explained facts offer an unexpected opportunity to widen the therapeutical applicability of thrombolytic agents in the treatment of acute ischaemic stroke.

As long as the process of infarction accompanying acute ischaemic stroke is not completed, it is throught that therapeutic treatment with a thrombolytic agent still has a positive benefit/risk ratio; since neuroprotectants such as lubeluzole are considered to present or delay the process of infarction from being completed, thus enlarging the inclusion period during which the combined preparation neuroprotectant/thrombolytic can be administered safely.

Hence, the present invention is concerned with a product containing
(a) a composition suitable for intravenous administration comprising a pharmaceutically effective amount of a neuroprotectant and a pharmaceutically acceptable carrier ; and
(b) a composition suitable for intravenous or intra-arterial administration, optionally after reconstitution with sterile water for injection, comprising a pharmaceutically effective amount of a thrombolytic agent and a pharmaceutically acceptable carrier,
as a combined preparation for simultaneous, separate or sequential use in the treatment of acute ischaemic stroke.

Hereinafter, the amounts of each of the ingredients in the compositions are expressed as percentages by weight based on the total volume of the formulation, unless otherwise indicated.

Neuroprotectants in particular are 2-aminobenzothiazole derivatives of formula (I)

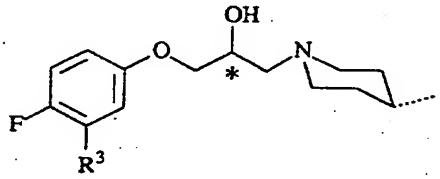


the pharmaceutically acceptable acid addition salts, the N-oxide forms, and the stereochemically isomeric forms thereof, wherein

R^1 represents hydrogen or C_{1-4} alkyl ;

R^2 represents hydrogen, hydroxy or trifluoromethoxy ; and

L represents hydrogen or a radical of formula (II)



wherein R^3 represents hydrogen or fluoro.

Particular neuroprotectants are lubeluzole (R^1 is methyl; R^2 is hydrogen, L is a radical of formula (II) wherein R^3 is fluoro and the atom marked * has the (S) configuration), lubeluzole N-oxide hemihydrate (oxidized piperidine nitrogen atom, preferably cis-oriented), 6-hydroxylubeluzole (R^2 is hydroxy) or riluzole (R^1 and L are both hydrogen, R^2 is trifluoromethoxy). The most preferred neuroprotectant is lubeluzole. Lubeluzole is generic to (S)-4-[(2-benzothiazolyl) methylamino]-α-[(3,4-difluorophenoxy)methyl]-1-piperidineethanol. Its preparation and properties are described in WO-92/14731.

Neuroprotectants may also be NMDA receptor antagonists such as aptiganel, eliprodil, selfotel, tirilazad or remacemide or combinations of the abovementioned 2-aminobenzothiazole derivatives of formula (I) therewith.

Pharmaceutically acceptable acid addition salts comprise the therapeutically active, non-toxic salt forms obtained by treating a base form with an acid such as, for example, an inorganic acid, e.g. hydrochloric, hydrobromic, sulfuric, nitric, phosphoric acid ; or an organic acid, e.g. acetic, propanoic, hydroxyacetic, lactic, pyruvic, malonic, succinic, maleic, fumaric, tartaric, citric, methanesulfonic, ethanesulfonic, benzene-sulfonic, *p*-toluenesulfonic, cyclamic, salicylic, *p*-aminosalicylic, pamoic acid.

The N-oxide forms of the compounds of formula (I) are meant to comprise those compounds wherein one or more of the nitrogen atoms are oxidized, in particular those wherein the piperidine nitrogen is oxidized. In lubeluzole N-oxide, the oxygen atom on the piperidine nitrogen is cis-oriented, i.e. to the same side of the plane defined by the piperidine ring as the 4-substituent.

The adsorption of lubeluzole to the walls of the *i.v.* administration equipment (infusor) can be reduced significantly by maintaining the pH of the solution (a) below 3.6. In this way, an intravenous solution can be prepared having superior physical stability.

The term "physically stability" or "physically stable" as used herein refers to a solution for which less than 10% by weight of active ingredient is adsorbed after passing through an infusion device. Preferably less than 5% by weight of active ingredient is adsorbed.

Generally, the pharmaceutically acceptable carrier for the neuroprotectant lubeluzole is an aqueous solution (a) comprising water ; an isotonizing agent ; and acid, base or buffer substances sufficient to adjust the pH of the solution in the range of from 2.5 to 3.6.

In particular, the concentration of lubeluzole in the present solutions (a) may range from 0.005% to 5%, preferably from 0.01% to 1%, more preferably from 0.02% to 0.2% and in particular is about 0.05%.

Further, the present solutions (a) conveniently comprise from 1 to 10% isotonizing agent. The use of glucose as isotonizing agent has the advantage that very clear solutions are obtained. Preferably, glucose is used in a concentration from 2 to 10%, most preferably of about 5%.

The solutions (a) further comprise acid and base substances to maintain the pH of the solution in the range from 2.5 to 3.6, preferably in the range from 3.0 to 3.4, most preferably at about 3.2. Preferably, the pH of the solutions (a) is adjusted by appropriate amounts of hydrochloric acid and sodium hydroxide. The pH may also be adjusted by buffer systems comprising mixtures of appropriate amounts of an acid such as phosphoric, tartaric or citric acid, and a base, in particular sodium hydroxide.

In order to increase the solubility of lubeluzole in the present formulations, a solubilizer may be used. Conveniently, a cyclodextrin (CD) or a derivative thereof may be used.

Appropriate cyclodextrin derivatives are α-, β-, γ-cyclodextrins or ethers and mixed ethers thereof wherein one or more of the hydroxy groups of the anhydroglucose units of the cyclodextrin are substituted with C_{1-6}alkyl, particularly methyl, ethyl or isopropyl, e.g. randomly methylated β-cyclodextrin; hydroxyC_{1-6}alkyl, particularly hydroxyethyl, hydroxypropyl or hydroxy-butyl; carboxyC_{1-6}alkyl, particularly carboxymethyl or carboxyethyl; C_{1-6}alkyl-carbonyl, particularly acetyl; C_{1-6}alkyloxycarbonylC_{1-6}alkyl or carboxyC_{1-6}alkyl-oxyC_{1-6}alkyl, particularly carboxymethoxypropyl or carboxyethoxy-propyl; C_{1-6}alkylcarbonyloxyC_{1-6}alkyl, particularly 2-acetyloxypropyl. Especially noteworthy as solubilizers are β-CD, 2,6-dimethyl-β-CD, randomly methylated β-cyclo-dextrin, 2-hydroxyethyl-β-CD, 2-hydroxyethyl-γ-CD, 2-hydroxypropyl-γ-CD and (2-carboxymethoxy)propyl-β-CD, and in particular 2-hydroxypropyl-β-CD.

The term mixed ether denotes cyclodextrin derivatives wherein at least two cyclodextrin hydroxy groups are etherified with different groups such as, for example, hydroxypropyl and hydroxyethyl.

The average molar substitution (M.S.) is used as a measure of the average number of moles of alkoxy units per mole of anhydroglucose. The M.S.value can be determined by various analytical techniques such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and infrared spectroscopy (IR). Depending on the technique used, slightly different values may be obtained for one given cyclodextrin derivative. In the cyclodextrin hydroxyalkyl derivatives for use in the compositions according to the present invention the M.S. as determined by mass spectrometry is in the range of 0.125 to 10, in particular of 0.3 to 3, or from 0.3 to 1.5. Preferably the M.S. ranges from about 0.3 to about 0.8, in particular from about 0.35 to about 0.5 and most particularly is about 0.4. M.S. values determined by NMR or IR preferably range from 0.3 to 1, in particular from 0.55 to 0.75.

The average substitution degree (D.S.) refers to the average number of substituted hydroxyls per anhydroglucose unit. The D.S. value can be determined by various analytical techniques such as nuclear magnetic resonance (NMR), mass spectrometry (MS) and infrared spectroscopy (IR). Depending on the technique used, slightly different values may be obtained for one given cyclodextrin derivative. In the cyclodextrin derivatives for use in the compositions according to the present invention the D.S. as determined by MS is in the range of 0.125 to 3, in particular of 0.2 to 2 or from 0.2 to 1.5. Preferably the D.S. ranges from about 0.2 to about 0.7, in particular from about 0.35 to about 0.5 and most particularly is about 0.4. D.S. values determined by NMR or IR preferably range from 0.3 to 1, in particular from 0.55 to 0.75.

More particular β– and γ-cyclodextrin hydroxyalkyl derivatives for use in the compositions according to the present invention are partially substituted cyclodextrin

derivatives wherein the average degree of alkylation at hydroxyl groups of different positions of the anhydroglucose units is about 0% to 20% for the 3 position, 2% to 70% for the 2 position and about 5% to 90% for the 6 position. Preferably the amount of unsubstituted β- or γ-cyclodextrin is less than 5% of the total cyclodextrin content and in particular is less than 1.5%. Another particularly interesting cyclodextrin derivative is randomly methylated β-cyclodextrin.

Most preferred cyclodextrin derivatives for use in the present invention are those partially substituted β-cyclodextrin ethers or mixed ethers having hydroxypropyl, hydroxyethyl and in particular 2-hydroxypropyl and/or 2-(1-hydroxypropyl) substituents.

The most preferred cyclodextrin derivative for use in the compositions of the present invention is hydroxypropyl-β-cyclodextrin having a M.S. in the range of from 0.35 to 0.50 and containing less than 1.5% unsubstituted β-cyclodextrin. M.S. values determined by NMR or IR preferably range from 0.55 to 0.75.

In order to minimize the risk of adverse reactions, an intravenous formulation preferably contains as few ingredients as possible. Therefore, a formulation without a solubilizer such as a cyclodextrin is preferred. It was found that the solubility of lubeluzole in formulations without a solubilizer ranges between about 9.2 mg/ml (pH 2.5) and about 2 mg/l (pH 3.6). Formulations of pH 3.2 without a solubilizer comprise at maximum about 3 mg/ml dissolved lubeluzole. Further, the neuroprotectant solution (a) preferably does not contain a preservative.

In particular, the present invention relates to neuroprotectant solutions (a) comprising:
(i) 0.005 to 5% lubeluzole or a pharmaceutically acceptable addition salt thereof;
(ii) 1 to 10% isotonizing agent;
(iii) acid and/or base substances to adjust the pH in the range from 2.5 to 3.6 ; and
(iv) water q.s. ad 100%.

Preferably, the invention relates to neuroprotectant solutions (a) comprising:
(i) 0.01 to 1% lubeluzole or a pharmaceutically acceptable addition salt thereof;
(ii) 2 to 10% glucose;
(iii) hydrochloric acid and sodium hydroxide to adjust the pH in the range from 3.0 to 3.4 ; and
(iv) water q.s. ad 100%.

Most preferably, the invention relates to neuroprotectant solutions (a) containing approximately :

(i) 0.05% lubeluzole or a pharmaceutically acceptable addition salt thereof;
(ii) 5% glucose;
(iii) hydrochloric acid and sodium hydroxide to adjust the pH to about 3.2; and
(iv) water q.s. ad 100%.

The solutions (a) are sterilized using art-known techniques.

The neuroprotectant solution (a) of the present product is conveniently used in the treatment of patients suffering from acute ischaemic stroke. In general it is contemplated that an effective treatment for acute ischaemic stroke involves administering to the patient first an amount of a neuroprotectant solution (a'), in particular lubeluzole, in the range of 10 to 30 ml of solution (a') or from 5 to 15 mg of lubeluzole during the first hour of therapy following the attendance by a physician. During the following 24 hours about 4/3 or 133 % of that amount in the form of solution (a) may be administered. That is, one starts with a relatively high initial flow which is then lowered considerably. The maintenance dose may be administered for several consecutive days.

Preferably about 15 ml of solution (a') or about 7.5 mg of lubeluzole is administered by infusion during the first hour of therapy, followed by about 20 ml of solution (a) or about 10 mg of lubeluzole during the next 24 hours. It is evident that said effective amount may be lowered or increased depending on the response of the treated subject and/or depending on the evaluation of the physician prescribing the compounds of the instant invention. The effective amount ranges mentioned hereinabove are therefore guidelines only and are not intended to limit the scope or use of the invention to any extent. The subject solutions may conveniently be co-administered with a physiological salt solution according to art-known procedures.

Thrombolytic agents in the products according to the present invention in particular are proteolytic enzyme plasmins selected from the group consisting of streptokinase, urokinase, pro-urokinase, tissue plasminogen activator (t-PA), fibrinolysin, staphylokinase and the like agents; most preferably tissue plasminogen activator (t-PA).

Generally, the thrombolytic agent composition (b) as used in clinical practice comprises water as the carrier and pharmaceutical adjuvants as known in the art, i.e. isotonizing agents; acid, base or buffer substances to adjust the pH of the solution; and stabilizing agents for the thrombolytic agent. Said thrombolytic agent composition (b) as stored

before use is preferably a lyophilized product which can be reconstituted with Sterile Water for Injection (USP).

The concentration of the thrombolytic agent in the lyophilized product depends on the nature of the thrombolytic agent. As an example, the thrombolytic agent tissue plasminogen activator (t-PA) will be considered in the following paragraphs.

Tissue plasminogen activator (t-PA) may be present in an amount of from 20 mg to 100 mg per dosage form (i.e. 11.6 million IU to 58 million IU). The concentration of tissue plasminogen activator (t-PA) in the lyophilized product is usually in the range of from 1.5 to 2% (w/w).

As pH adjusting agents, phosphoric acid and optionally sodium hydroxide may be used, so that upon reconstitution with sterile water for injection, a pH of about 7.3 is reached; in particular the lyophilized product comprises from 15 to 25% (w/w) of phosphoric acid. As stabilizing agent for the thrombolytic agent, it is convenient to use an amino acid, for example, L-arginine in the case of tissue plasminogen activator (t-PA). The stabilizing agent makes up the bulk of the lyophylized thrombolytic agent, typically from about 70% to about 80% (w/w).

In particular, the present invention relates to tissue plasminogen activator (t-PA) compositions (b) comprising in the lyophilized state :

(v) from 1.5 to 2% (w/w) t-PA;
(vi) from 15 to 25% (w/w) phosphoric acid; and
(vii) from 70 to 80% (w/w) L-arginine.

Further adjuvants in the compositions (b) are, for example, wetting agents, e.g. polysorbate, in particular polysorbate 80 which may be used in a concentration up to 1%, more in particular up to 0.85%.

The thrombolytic agent according to the present invention is conveniently used in stroke patients in the following manner. The lyophilized product comprising 20, 50 or 100 mg t-PA per dosage form (vial) is reconstituted with sterile water for injection, in the case of tissue plasminogen activator (t-PA), for example, to a solution having a concentration of 1 mg/ml.

The total dose of thrombolytic agent, in particular of t-PA will be at most 0.95 mg/kg bodyweight, preferably about 0.90 mg/kg bodyweight, with an absolute upper limit of 100 mg; preferably an upper limit of 90 mg.

Tissue plasminogen activator is administered to the stroke patient in the following dose regimen : up to 10% of total dose is administered as a bolus, the remaining 90% then being administered as a constant infusion during the next hour. The administration of the thrombolytic agent will occur simultaneously with the administration of the neuroprotectant solution (a), which -it should be noted- will be continued after the administration of the thrombolytic agent has been completed.

The neuroprotectant and thrombolytic solutions may conveniently be co-administered with a physiological salt solution following to art-known infusion procedures.

Preferred products according to the present invention are those wherein the neuroprotectant solution (a) and the thrombolytic preparation (b) are miscible and - when mixed - form a stable formulation for up to eight hours at room temperature. The two formulations can then be stored together, but in separate containers such as vials, pre-filled syringes and the like, and mixed immediately before use. A preferred container comprises the neuroprotectant solution (a) and thrombolytic preparation (b) separately in a two-chamber container including means to mix both liquids. The two-chamber container ideally is a pre-filled, two-chamber syringe with bypass or similar means (e.g. a breakable seal) allowing mixing of the two separate solutions prior to administration, and which is further adapted for use with infusor devices.

A specific product is adapted for administering a neuroprotectant solution (a) and a thrombolytic preparation (b) following an hour of neuroprotectant therapy by an attending physician, and within three to six hours of the occurence of the ischaemic event. If the neuroprotectant is the preferred active ingredient lubeluzole, then the product comprises about 20 ml of solution or about 10 mg of lubeluzole.

The present invention evidently also concerns the use of a product as described hereinbefore for the preparation of a medicament for acute ischaemic stroke treatment. Similarly, the present invention relates to a method of treating patients suffering from acute ischaemic stroke, comprising administering simultaneously, separately or sequentially to said patients the components of a product as described hereinbefore.

The present invention also concerns the use of a neuroprotectant for the preparation of a medicament for preventing or delaying the process of infarction accompanying acute ischaemic stroke from being completed, thus enlarging the inclusion period during which the combined preparation of neuroprotectant/thrombolytic agent can be administered safely.

Experimental part

Example 1 : Preparation of (-)-[cis] lubeluzole N-oxide hemihydrate.

To a stirred solution of lubeluzole (11.6 g ; 27 mmol) in dichloromethane (700 ml), cooled to -10°C, was added m-chloroperbenzoic acid (6.7 g ; 31 mmol). The reaction mixture was stirred for 24 hours, and then washed with an aqueous ammonia solution (2% ; 3 times) and water (3 times). The organis phase was dried on MgSO4, filtered and evaporated, yielding 9.6 of raw material. The product (-)-[cis] lubeluzole N-oxide hemihydrate was purified by recrystallization from methylisopropylketone (mp. 182.8°C) (yield : 4.7 g ; 38.7%) $[\alpha]_D^{20}$ = -8.73° (1% in methanol) (comp. 1).

Example 2 : Preparation of 6-hydroxylubeluzole.

a) Ethyl 4-(methylamino)-1-piperidinecarboxylate (59 mmol), 2-chloro-6-methoxy-benzothiazole (49 mmol) and sodium carbonate (50 mmol) were stirred at 180°C under N_2 flow on an oil bath overnight. The mixture was cooled to room temperature. $CHCl_3$ was added. The mixture was stirred on in ultrasonic bath for 10 minutes and then washed twice with water and once with a NaCl 50% solution. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (eluent: $CHCl_3$/ hexane/ CH_3OH 50/49/1). The pure fractions were collected and the solvent was evaporated, yielding 16g (93.5%) of ethyl 4-[(6-methoxy-2-benzothiazolyl)-methylamino]-1-piperidinecarboxylate (interm. 1).

b) A mixture of intermediate (1) (50.4 mmol) in a solution of hydrobromic acid in water (140ml) was stirred and refluxed overnight. The mixture was cooled, evaporated and the residue was boiled in 2-propanol. After cooling to room temperature, the precipitate was collected by filtration, washed with 2-propanol and with 2,2'-oxybispropane and was then dried on the air. The precipitate was dissolved in water (200ml) and basified till pH 9.3 with NH_4OH. The aqueous layer was decanted, the oily residue was stirred 3 times in a bit water and decanted. The oily residue was boiled in CH_3CN (80ml), the precipitate was filtered off at room temperature and dried, yielding 9.1g (71%) of 2-(methyl-4-piperidinylamino)-6-benzothiazolol; mp. 222.9°C (interm. 2).

c) A mixture of (+)-(S)-[(3,4-difluorophenoxy)methyl]oxirane (3.22 mmol) and intermediate (2) (3.2 mmol) in 1-butanol (10ml) was stirred for 22 hours at 125°C (oil

bath). The reaction mixture was cooled to room temperature and the solvent was evaporated. The residue was purified by column chromatography over silica gel (24x160mm;eluent: $CHCl_3/CH_3OH$/n-hexane 45/10/45). The pure fractions were collected and the solvent was evaporated. The residue (2.458 mmol) was dissolved in 2-propanol (20ml) and converted into the (Z)-2-butenedioic acid salt (1:2) with a solution of (Z)-2-butenedioic acid (4.92 mmol) in 2-propanol (10ml). The mixture was heated for 2 minutes. The crystals were filtered off at room t° and dried. Yielding: 1.471g (67.4%) of (-)-(S)-2-[[1-[3-(3,4-difluorophenoxy)-2-hydroxypropyl]-4-piperidinyl]-methylamino]-6-benzothiazolol (Z)-2-butenedioate (1:2); mp. 180.2°C; $[\alpha]_D^{20}$ = - 8.03° (c = 1% in methanol) (comp. 2).

Pharmacological examples

The useful anti-hypoxic properties of the products of the present invention can be demonstrated in the following test procedure.

Example 3 : Post-Treatment in a Rat Photochemical Stroke Model.

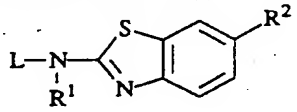
Male Wistar rats, weighing 260-280 g, are anesthetized with halothane in a N_2O/O_2 mixture. The animals are placed in a stereotactic apparatus, the scalp is incised for exposure of the skull surface, and a catheter is inserted into a lateral tail vein. Rose Bengal (30mg/kg; 15 mg/ml in 0.9% NaCl) is infused intravenously for 2 minutes in animals with normal hemodynamics and blood gases. Thereafter, the skull is focally illuminated with cold white light for 5 minutes by means of a fiber-optic bundle inside a 1-mm diameter objective. The light is aimed at the hindlimb area of the right parietal sensorimotor neocortex. Five minutes after infarct induction (i.e. 5 min after light offset), the rats are injected with the product.

Neurologic tests, involving limb placing reactions, are conducted on the first two days after infarction at 24-hour intervals after its induction.Tactile forward and sideways placing are tested by lightly contacting the table edge with the dorsal or lateral aspect of a paw (2 tests). Proprioceptive forward and sideways placing involves pushing the paw against the table edge in order to stimulate limb muscles and joints (2 tests). Rats are also put along the edge of an elevated platform in order to assess proprioceptive adduction : a paw is gently pulled down and away from the platform edge, and, upon sudden release, it is checked for retrieval and placing (1 test). For each of the 5 tests, placing scores are : 0, no placing; 1, incomplete and/or delayed placing; or 2, immediate, complete placing. For each limb, the summed tactile/proprioceptive placing score, including the platform test, is maximally 10. Results are reported from the deficient hindlimb contralateral to the neocortical infarct. Six rats are used for each dose.

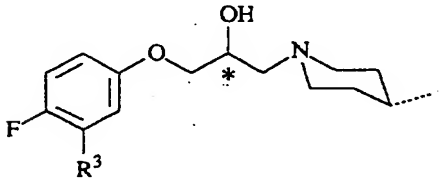
Claims

1. A product containing
   (a) a composition suitable for intravenous administration comprising a pharmaceutically effective amount of a neuroprotectant and a pharmaceutically acceptable carrier ; and
   (b) a composition suitable for intravenous or intra-arterial administration, optionally after reconstitution with sterile water for injection, comprising a pharmaceutically effective amount of a thrombolytic agent and a pharmaceutically acceptable carrier, as a combined preparation for simultaneous, separate or sequential use in the treatment of acute ischaemic stroke.

2. A product according to claim 1 wherein the neuroprotectant is a 2-amino-benzothiazole derivative of formula (I)



a pharmaceutically acceptable acid addition salt, an N-oxide form or a stereochemically isomeric form thereof, wherein
R^1 represents hydrogen or C_{1-4} alkyl ;
R^2 represents hydrogen, hydroxy or trifluoromethoxy ; and
L represents hydrogen or a radical of formula



wherein R^3 represents hydrogen or fluoro.

3. A product according to claim 2 wherein the neuroprotectant is lubeluzole, lubeluzole N-oxide, 6-hydroxylubeluzole or riluzole.

4. A product according to claim 3 wherein the neuroprotectant lubeluzole is formulated in an aqueous solution comprising water ; an isotonizing agent ; and acid, base or buffer substances sufficient to adjust the pH of the solution in the range of from 2.5 to 3.6.

5. A product according to claim 4 wherein the neuroprotectant solution (a) comprises :
   (i) 0.005 to 5% lubeluzole or a pharmaceutically acceptable addition salt thereof;
   (ii) 1 to 10% isotonizing agent;
   (iii) acid and/or base substances to adjust the pH in the range from 2.5 to 3.6 ; an
   (iv) water q.s. ad 100%.

6. A product according to claim 5 wherein the neuroprotectant solution (a) comprises :
   (i) 0.01 to 1% lubeluzole or a pharmaceutically acceptable addition salt thereof;
   (ii) 2 to 10% glucose;
   (iii) hydrochloric acid and sodium hydroxide to adjust the pH in the range from 3.0 to 3.4 ; and
   (iv) water q.s. ad 100%.

7. A product according to claim 6 wherein the neuroprotectant solution (a) comprises :
   (i) 0.05 % (w/v) lubeluzole or a pharmaceutically acceptable acid addition salt thereof ;
   (ii) 5 % (w/v) glucose ;
   (iii) hydrochloric acid and sodium hydroxide to adjust the pH to about 3.2 ; and
   (iv) water q.s. ad 100 %.

8. A product according to claim 7 wherein the neuroprotectant solution (a) is present in an amount of from 10 to 30 ml, and comprises about 5 to 15 mg lubeluzole.

9. A product according to claim 8 wherein the neuroprotectant solution (a) is present in an amount of about 20 ml, and comprises about 10 mg lubeluzole.

10. A product according to anyone of claims 1 to 9 comprising a second neuroprotectant solution (a') which is present in an amount of about 15 ml, and comprises about 7.5 mg lubeluzole.

11. A product according to any one of claims 1 to 10 wherein the thrombolytic agent is a proteolytic enzyme plasmin selected from the group consisting of streptokinase, urokinase, pro-urokinase, tissue plasminogen activator (t-PA), fibrinolysin, staphylokinase and the like agents.

12. A product according to claim 11 wherein the thrombolytic agent is tissue plasminogen activator (t-PA).

13. A product according to claim 11 wherein the thrombolytic agent tissue plasminogen activator (t-PA) is in a lyophilized form which can be reconstituted with sterile water for injection.

14. A product according to claim 13 wherein the thrombolytic agent composition (b) comprises in the lyophilized state :
    (v) from 1.5 to 2% (w/w) t-PA;
    (vi) from 15 to 25% (w/w) phosphoric acid; and
    (vii) from 70 to 80% (w/w) L-arginine.

15. A product according to claim 13 further comprising a wetting agent.

16. A product according to claim 13 comprising 20, 50 or 100 mg of tissue plasminogen activator (t-PA).

17. A product according to any one of claims 1 to 16 wherein the neuroprotectant solution (a) and the thrombolytic preparation (b) are miscible and - when mixed - form a stable formulation for up to eight hours at room temperature.

18. A product according to claim 17 wherein the neuroprotectant solution (a) and the thrombolytic preparation (b) are contained separately in a two-chamber container, including means to mix both liquids.

19. A product according to claim 18 wherein the two-chamber container is a two-chamber syringe with bypass or similar means allowing mixing of the two separate solutions prior to administration, and which is further adapted for use with infusor devices.

20. The use of a product as claimed in any of claims 1 to 19 for the preparation of a medicament for the treatment of conditions involving acute ischaemic stroke.

21. The use of a neuroprotectant for the preparation of a medicament for preventing or delaying the process of infarction accompanying acute ischaemic stroke from being completed, thus enlarging the inclusion period during which the combined preparation of neuroprotectant/thrombolytic agent can be administered safely.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 96/04609

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/49 A61K38/48 A61K31/445 A61K31/425

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	ARCH NEUROL, 48 (12). 1991. 1235-1238., XP000610853 ZIVIN J A ET AL: "TISSUE PLASMINOGEN ACTIVATOR PLUS GLUTAMATE ANTAGONIST IMPROVES OUTCOME AFTER EMBOLIC STROKE" see abstract ---	1
X	SCHWEIZ RUNDSCH MED PRAX, SEP 19 1995, 84 (38) P1025-31, SWITZERLAND, XP000610911 HOMMEL M ET AL: "[Thrombolytic therapy in ischemic cerebrovascular accidents]" see page 1030, column 1, paragraph 2 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
12 February 1997	28.02.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Leherte, C

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INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCI/EP 96/04609

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	NEUROLOGICAL RESEARCH, 15 (5). 1993. 344-349., XP000610900 OVERGAARD K ET AL: "Neuroprotection with NBQX and thrombolysis with rt-PA in rat embolic stroke" see abstract	1
A	WO 94 12478 A (SMITHKLINE BEECHAM CORP ;KEENAN RICHARD MCCULLOCH (US); MILLER WIL) 9 June 1994 see page 2, line 36 - page 4, line 16 see page 22, line 6 - line 21	1-21
A	US 5 434 150 A (AUSTEL VOLHARD ET AL) 18 July 1995 see column 1, line 1 - column 4, line 45 see column 24, line 33 - column 25, line 7	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte- ional Application No
PCI/EP 96/04609

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		EP-A-	0531883	17-03-93
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		JP-A-	6025181	01-02-94
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